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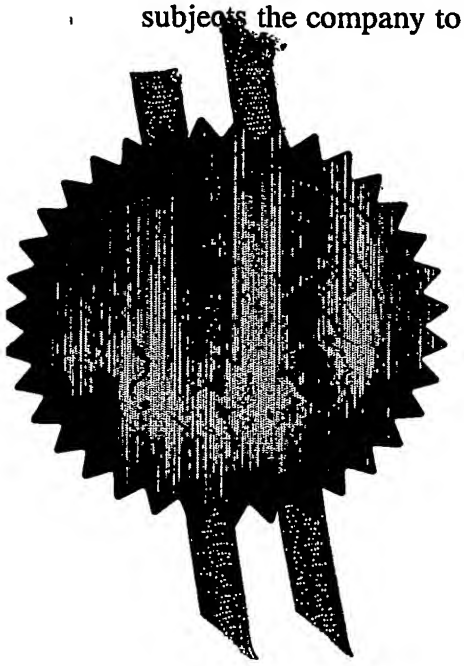

Signed

Dated 14 January 2004

Patents Form 1/77

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THE PATENT OFFICE
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19NOV02 E643537-1 D00056
P01/7700 0.00-0226855.5

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP9 1RH

1. Your reference — P34922GB/NCB

2. Patent application number *(The Patent Office will fill in this part)* — 0226855.5 — 18 NOV 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Queen Mary & Westfield College
Mile End Road
London E1 4NS
GB 6192033001

University College London
Gower Street
London WC1E 6BT
GB 7986520002

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — GB — GB

4. Title of the invention

HISTONE DEACETYLASE INHIBITORS

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Kilburn & Strode
20 Red Lion Street
London
WC1R 4PJ

Patents ADP number *(if you know it)* — 125001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	31 ✓
Claim(s)	7 ✓
Abstract	-
Drawing(s)	2 + 2 ✓

NSC

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature: Kilburn & Strode

Date: 18 November 2002

Kilburn & Strode

12. Name and daytime telephone number of person to contact in the United Kingdom

Mr Nick C Bassil
Tel: 020 7539 4200

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After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

e) *Once you have filled in the form you must remember to sign and date it.*

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HISTONE DEACETYLASE INHIBITORS

The present invention relates to histone deacetylase inhibitors, methods for the synthesis of such compounds, use of the compounds in medicine.

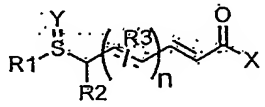
A number of recent research reports suggest that chromosome translocations in cancer cells disrupt proteins involved in the process of histone acetylation and de-acetylation, and that these abnormal proteins cause aberrant gene repression. Histones are the protein component of chromatin, which comprises DNA supported by histone octamers to form nucleosomes. These histone proteins have lysine rich tails which when deacetylated become charged and attracted to the DNA backbone. This condenses the chromatin structure such that proteins involved in gene transcription cannot gain access, resulting in transcriptional repression.

It has been proposed that inhibition of histone deacetylase (HDAC) enzymes could relieve such gene repression and reinstate the program of differentiation and apoptosis in a manner analogous to the use of retinoic acid in the treatment of acute promyelocytic leukemia - a form of "transcription therapy" A number of compounds that inhibit HDAC have been described, and several are in phase I and II clinical trials. These compounds have been shown to induce cell cycle arrest, differentiation and cell death in cancer cells growing *in vitro* and in animal xenograft models. The most potent HDAC inhibitor, Trichostatin A (TSA) was isolated from *Streptomyces hygroscopicus* in the 1970's, as an antifungal antibiotic against *trichophyton.* Although potent *in vitro,* TSA has limited stability and is therefore not therapeutically useful. Novel compounds with a similar structure, such as suberoylanilide hydroxamate (SAHA), have activity in pre-clinical models, and have shown some anti-cancer activity in phase I studies. However, this compound is also rapidly eliminated, requiring large doses for activity. Other HDAC inhibitors that have been tested in the phase I setting have major side effects (i.e. Depsipeptide), or affect histone acetylation by an indirect mechanism (CI-994). Others are still undergoing early clinical investigation.

Potent, metabolically stable, HDAC inhibitors would be more therapeutically useful than many of those currently in clinical trials.

A new class of compounds that are inhibitors of HDAC has now been prepared which are believed to be more metabolically stable and more robust to *in vivo* enzyme attack, in which the compounds are characterised by the presence of an *isostere* $S(=O)_n$ (in place of C=O in trichostatin (TSA)).

According to a first aspect of the invention, there is provided a compound of general formula (1):



1

in which:

R^1 may be (C_6 or C_{10}) aryl, (C_6 or C_{10}) arylalkyl, (C_6 or C_{10}) heteroaryl, (C_3-C_8) heterocycloalkenyl, (C_5-C_8) cycloalkene ring, (C_5-C_8) cycloalkyl, (C_5-C_8) heterocycloalkyl or a combination thereof to form a linked or fused ring system, the cyclic moiety being optionally substituted with (C_1-C_{10}) alkyl, (C_1-C_{10}) alkenyl, (C_1-C_{10}) alkynyl, (C_1-C_{10}) alkoxy, (C_1-C_{10}) thioalkoxy, hydroxyl, hydroxyl, (C_1-C_{10}) hydroxylalkyl, halo, (C_1-C_{10}) haloalkyl, amino, amido, (C_1-C_{10}) alkylcarbonyloxy, (C_1-C_{10}) alkoxycarbonyl, (C_1-C_{10}) alkylcarbonyl, (C_1-C_{10}) alkylthiocarbonyl, (C_1-C_{10}) alkylsulfonylamino, aminosulfonyl, (C_1-C_{10}) alkylsulfinyl, or (C_1-C_{10}) alkylsulfonyl,

R^2 and R^3 may each independently be hydrogen, (C_1-C_6) alkyl, substituted (C_1-C_6) alkyl, or unsaturated (C_1-C_6) comprising one or more C=C bond or C≡C bond, (C_6 or C_{10}) aryl or (C_6 or C_{10}) heteroaryl, or a combination thereof to form a linked or fused ring system, or (C_1-C_{10}) alkyl, (C_1-C_{10}) alkenyl, (C_1-C_{10}) alkynyl, (C_1-C_{10}) alkoxy, (C_1-C_{10}) thioalkoxy, hydroxyl, hydroxyl, (C_1-C_{10}) hydroxylalkyl, halo, (C_1-C_{10}) haloalkyl, cyano, nitro, amino, amido, (C_1-C_{10}) alkylcarbonyloxy, (C_1-C_{10})

alkoxycarbonyl, (C_1-C_{10}) alkylcarbonyl, (C_1-C_{10}) alkylthiocarbonyl, (C_1-C_{10}) alkylsulfonylamino, aminosulfonyl, (C_1-C_{10}) alkylsulfinyl, or (C_1-C_{10}) alkylsulfonyl, or a saturated C3-C12 hydrocarbon chain or an unsaturated C_3-C_{12} hydrocarbon chain optionally interrupted by O, S, NR, CO, C(NR), $N(R)SO_2$, $SO_2N(R)$, N(R)C(O)O, OC(O)N(R), N(R)C(O)N(R), OC(O), C(O)O, OSO_2, SO_2O, or OC(O)O, where R may be independently hydrogen, (C_1-C_{10}) alkyl. (C_1-C_{10}) alkenyl, (C_1-C_{10}) alkynyl, (C_1-C_{10}) alkoxy, (C_1-C_{10}) hydroxylalkyl, hydroxyl, (C_1-C_{10}) halolalkyl, where each of the saturated or unsaturated hydrocarbon chains may be optionally substituted with (C_1-C_{10}) alkyl, (C_1-C_{10}) alkenyl, (C_1-C_{10}) alkynyl, (C_1-C_{10}) alkoxy, hydroxyl, hydroxyl, (C_1-C_{10}) hydroxylalkyl, halo, (C_1-C_{10}) haloalkyl, amino, (C_1-C_{10}) alkylcarbonyloxy, (C_1-C_{10}) alkoxycarbonyl, (C_1-C_{10}) alkylcarbonyl, (C_1-C_{10}) alkylsulfonylamino, aminosulfonyl, or (C_1-C_{10}) alkylsulfonyl,

or R^2 and R^3 optionally form a fused ring system together,

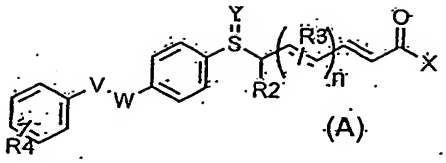
n may be equal to 0, 1 or 2,

X may be hydroxyl (-OH), hydroxamate (-NHOH), $NHOR^4$, NR^5OR^4, NR^5NHR^6,

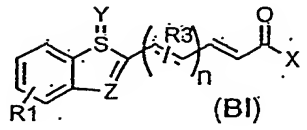
where R^4, R^5 or R^6 may each independently be hydrogen, C_1-C_6 alkyl or substituted C_1-C_6 alkyl, and

Y may be 0, 1 or 2 oxygen atoms, or NR^7 where R^7 may be OH, OR^8 or a carbon atom, where R^8 may be C_1-C_6 alkyl or substituted C_1-C_6 alkyl.

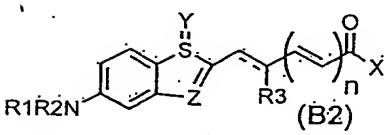
The compounds of general formula (1) may also include a *saturated linkage* (with or without substituents, replacing CH=CH in structure 1) adjacent to the COX group. Additionally, the chain linkage (as a whole or in part) may be of any level of saturation, and may incorporate rings fused anywhere onto the chain linking the end group and the COX terminus.



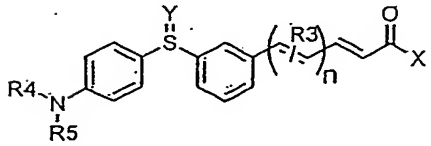
(A)



(B1)



(B2)



(C)

The compounds of general formula (1) may also include more specific classes of types (A) with the scope of substituents and rings *etc.* as outlined for **1**. In particular, V and W may constitute a single bond between the aromatic (or heterocyclic or alicyclic) rings, or they may take the form V = CR and W = N (such that a linkage RC=N is present), or the form V = N and W = CR (such that a linkage N=CR is present), or a saturated version of either of those linkages (with or without alkyl aryl, heterocyclic, or other substituents) or a linkage of the form VW or WV = R^5R^6C-O or R^5R^6C-S. Additionally, the chain linkage attached to COX may be of any level of saturation, and may incorporate rings fused onto the chain linking the end group and the COX terminus.

The compounds of general formula (1) may also include more specific classes of types (**B1**) and especially to types (**B2**) both with the scope of substituents and rings

alreadys as defined for 1 above, and n = zero, one or two. However, especially noteworthy for type (**B2**) are Y = no atom, O or O_2 or NR and Z = CR or N and n = zero, one or two and X = NHOH, OH, NR^5OR^4, CR^4R^5OH or derivatives or related groups for X, including any combination of the aforementioned groups and substituents for X, Y and Z. Additionally, the chain linkage may be of any level of saturation, and may incorporate rings fused onto the chain linking the end group and the COX terminus. For both (**B1**) and (**B2**), Z may also be a two-atom linkage of varying combinations of atoms, in particular C, O, N, S, SO, SO_2 and forming part of a saturated, partly saturated, or unsaturated ring.

The compounds of general formula (**1**) may also include more specific classes of types (**C**), in which Y = no atom, O or O_2 or NR and n = zero, one or two and X = NHOH, OH, NR^5OR^4, CR^4R^5OH or derivatives or related groups for X, including any combination of the aforementioned groups and substituents for X, Y and Z (although not herein covered by experimental details). Additionally, the chain linkage may be of any level of saturation, and may incorporate rings fused onto the chain linking the end group and the COX terminus.

Definitions:

In this specification the term "compound" includes "salt" or "hydrate" unless the context requires otherwise.

As used herein the term "halogen" or its abbreviation "halo" means fluoro, chloro, bromo or iodo.

As used herein the term "hetero" refers to the presence of one or more atoms that are not carbon atoms. Suitable heteroatoms include, oxygen, sulphur, nitrogen or phosphorus, represented as O, S, N and P, respectively.

As used herein the term "(C_1-C_6) alkyl" refers to straight chain or branched chain hydrocarbon groups having from one to six carbon atoms. Illustrative of such alkyl

groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *sec*-butyl, *tert*-butyl, pentyl, neopentyl, and hexyl. From one to four carbon atoms may be preferred.

As used herein the term "(C_1-C_{10}) alkyl" refers to straight chain or branched chain hydrocarbon groups having from one to ten carbon atoms. Illustrative of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *sec*-butyl, *tert*-butyl, pentyl, neopentyl, hexyl, heptyl, octyl, nonyl and decyl. From one to six carbon atoms may be preferred.

The presence of a partial carbon-carbon bond is indicated in General Formula (1) and should be intepreted as showing either a full double bond or a single bond (in which case hydrogen atoms are included to make up the full valency of carbon).

The term "(C_6 or C_{10})aryl" includes phenyl and naphthyl.

As used herein, the term "(C_5-C_8)cycloalkyl" refers to an alicyclic group having from 5 to 8 carbon atoms. Illustrative of such cycloalkyl groups are cyclopentyl and cyclohexyl.

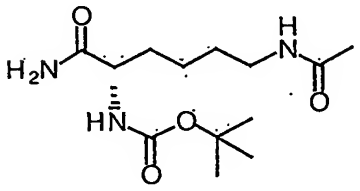
As used herein, the term "(C_5-C_8)cycloalkene ring" refers to an alicyclic ring having from 5 to 8 atoms and having in addition one or more double bonds. Illustrative of such cycloalkenyl groups are cyclopentenyl, cyclohexenyl, cycloheptenyl and cyclooctenyl.

In compounds of this invention, the presence of an asymmetric carbon atom gives rise to enantiomers. The presence of several asymmetric carbon atoms give rise to diastereoisomers, each of which consists of two enantiomers, with the appropriate R or S stereochemistry at each chiral centre. The invention is understood to include all such diastereoisomers, optically active enantiomers and mixtures thereof.

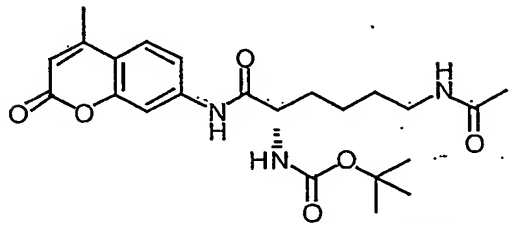
The term "suitable salt" refers to a salt prepared by contacting a compound of formula I with an acid or base whose counterpart ion does not interfere with the intended use of the compound. Examples include the sodium salt or magnesium salt of a phosphate

derivative or the salt formed from a primary, secondary or tertiary amine where the compound of general formula I is a carboxylic acid. An example of a primary amine salt can be the cyclohexylammonium salt, a suitable secondary amine salt may be the piperidine salt and a tertiary amine salt may be the triethylamine salt.

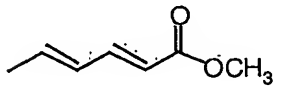
Preferred compounds of general formula I include those in which, independently or in any compatible combination:



(5-Acetylamino-1-carbamoyl-pentyl)-carbamic acid *tert*-butyl ester

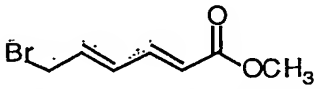


[(S)-5-Acetylamino-1-(4-methyl-2-oxo-2H-chromen-7-ylcarbamoyl)-pentyl]-carbamic acid-*tert*-butyl ester



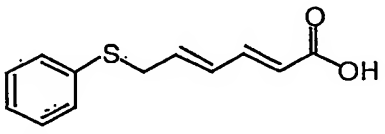
3a

Hexa-2,4-dienoic acid methyl ester



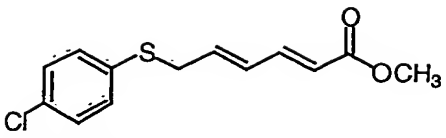
4a

6-Bromo-hexa-2,4-dienoic acid methyl ester



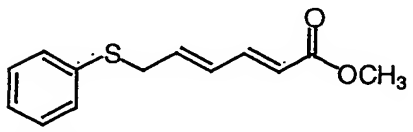
6a

6-Phenylsulfanyl-hexa-2,4-dienoic acid



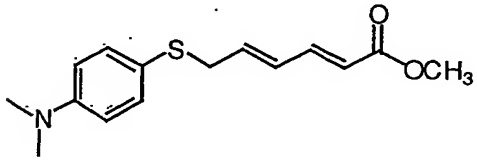
6b

6-(4-Chloro-phenylsulfanyl)-hexa-2,4-dienoic acid methyl ester

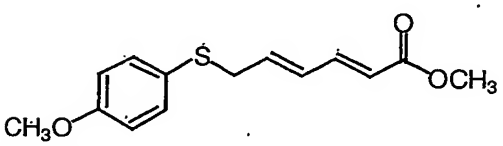


6c

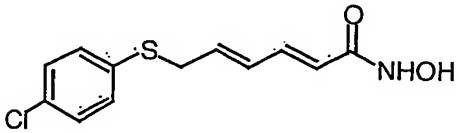
6-Phenylsulfanyl-hexa-2,4-dienoic acid methyl ester



6-(4-Dimethylamino-phenylsulfanyl)-hexa-2,4-dienoic acid methyl ester **6d**

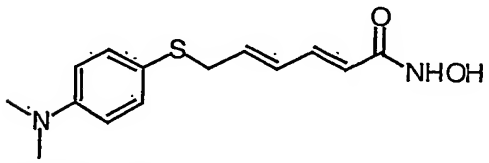


6-(4-Methoxy-phenylsulfanyl)-hexa-2,4-dienoic acid methyl ester **6e**

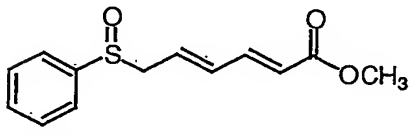


7b

6-(4-Chloro-phenylsulfanyl)-hexa-2,4-dienoic acid hydroxyamide

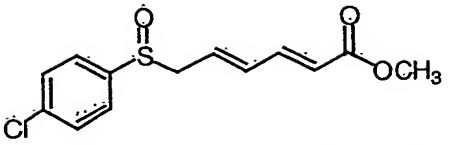


6-(4-Dimethylamino-phenylsulfanyl)-hexa-2,4-dienoic acid hydroxyamide **7c**



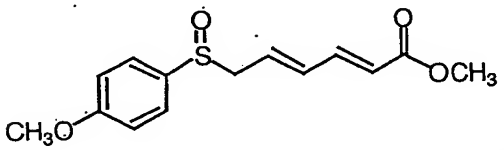
8a

6-Phenylsulfinyl-hexa-2,4-dienoic acid methyl ester



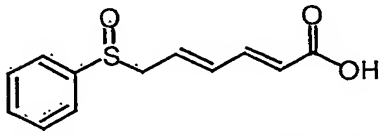
8b

6-(4-Chloro-benzenesulfinyl)-hexa-2,4-dienoic acid methyl ester

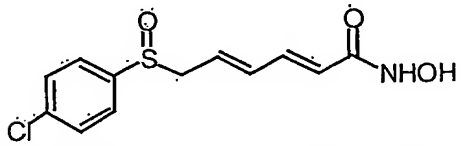


8c

6-(4-Methoxy-benzenesulfinyl)-hexa-2,4-dienoic acid methyl ester

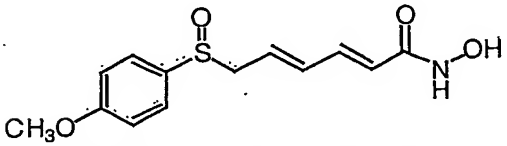


6-Benzenesulfinyl-hexa-2,4-dienoic acid **8d**



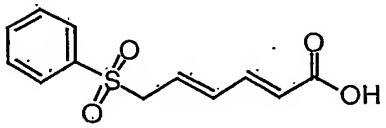
9a

6-(4-Chloro-benzenesulfinyl)-hexa-2,4-dienoic acid hydroxyamide



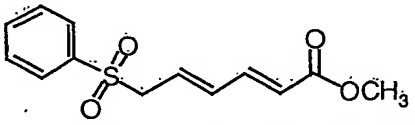
9b

6-(4-Methoxy-benzenesulfinyl)-hexa-2,4-dienoic acid hydroxyamide



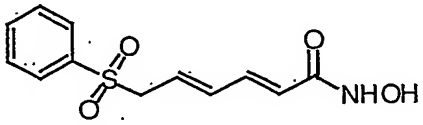
10a

6-Benzenesulfonyl-hexa-2,4-dienoic acid

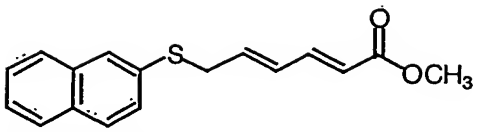


10b

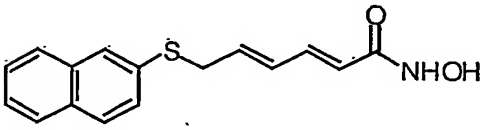
6-Benzenesulfonyl-hexa-2,4-dienoic acid methyl ester



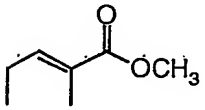
6-Benzenesulfonyl-hexa-2,4-dienoic acid hydroxyamide **11a**



6-(Naphthalen-2-ylsulfanyl)-hexa-2,4-dienoic acid methyl ester **13b**

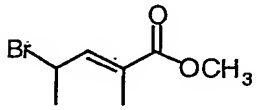


6-(Naphthalen-2-ylsulfanyl)-hexa-2,4-dienoic acid hydroxyamide **14a**



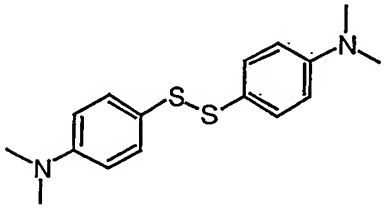
16b

2-Methyl-pent-2-enoic acid methyl ester



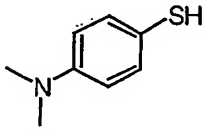
17b

4-Bromo-2-methyl-pent-2-enoic acid methyl ester

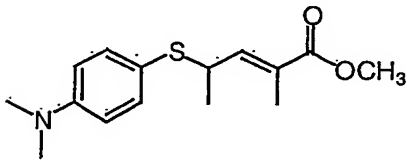


19

4-(N,N-dimethylamino)phenyl disulfide

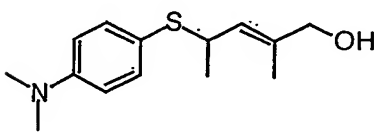


4-Dimethylamino-benzenethiol **20**



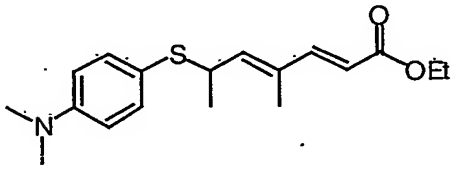
21b

4-(4-Dimethylamino-phenylsulfanyl)-2-methyl-pent-2-enoic acid methyl ester



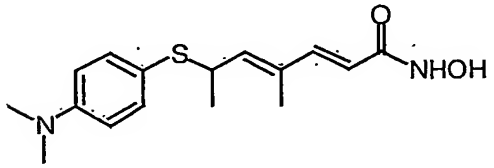
22b

4-(4-Dimethylamino-phenylsulfanyl)-2-methyl-pent-2-en-1-ol



24c

6-(4-Dimethylamino-phenylsulfanyl)-4-methyl-hepta-2,4-dienoic acid ethyl ester



25c

6-(4-Dimethylamino-phenylsulfanyl)-4-methyl-hepta-2,4-dienoic acid hydroxyamide

According to a second aspect of the invention there is provided a process for the preparation of a compound of general formula (**1**), comprising the addition of a compound of general formula (**5**) to general formula (**4**), optionally followed by further derivatisation.

According to a third aspect of the invention, there is provided a process for the preparation of a compound of general formula (**1**), comprising the addition of a compound of general formula (**20**) to a compound of general formula (**17**).

Synthetic Routes.

In all Schemes and in the experimental section, all the C=C double bonds that form part of the chain attached to the hydroxamic acid were isolated as the *trans*-isomer or *trans-trans* isomer (R^1 and R^2, or R^2 and R^3 are ignored for the purpose of the stereochemical description '*trans*' used above). In Scheme 1 is shown a general route to hydroxamic acid derivatives containing a sulfide (type **7**), a sulfoxide (type **9**) and a sulfone (type **11**) linkage. Those types can be accessed through a sequence involving addition of a thiol **5** to an unsaturated bromo ester **4** (or similar chloro or iodo derivative) to give an ester **6** which can be oxidised to **8** using sodium metaperiodate (or similar oxidising agent such as hydrogen peroxide). The corresponding sulfones **10** can be prepared either by oxidation of **6** or **8**, usually using a peracid, such as

meta-chloroperoxybenzoic acid. Reaction of **6**, **8** or **10** with hydroxylamine (usually an aqueous solution, but otherwise a salt such as hydroxylamine hydrochloride together with a base, typically sodium hydroxide or potassium hydroxide). The unsaturated bromo esters **4** were prepared by bromination of the corresponding esters **3**, usually using *N*-bromosuccinimide together with a sun lamp of 250 W (*i.e.* in the range 100 to 500 W for small-scale reactions). Such brominations can also be performed using a peroxide initiator such as dibenzoyl peroxide. The esters **3** can be conveniently prepared by treating the corresponding alcohol **2** with the appropriate alcohol (ROH) in the presence of sulfuric acid (or other catalyst).

Although a sequence of general utility is implied in Scheme 1, compounds of particular interest include R^2=R^3=H; R^2=Me, R^3=H; R^2=H, R^3=Me and R^2=R^3=Me for the set of compounds **2** - **4** and **6** - **11**, **13** and **14**.

Scheme 1 is intended to include aromatic and heteroaromatic rings, single, fused or poly-condensed ring systems without limitation (in place of the single benzene ring shown for compounds **5** – **11**) and with or without a wide variety of substituents. As an example, 2-naphthalenethiol **12** was shown to react with bromo ester **4** to give ester **13** which was reacted with aqueous hydroxylamine to give hydroxamic acid **14**. Hydroxamic acids of type **7**, **9**, **11** and **14** are of particular interest as inhibitors of histone deacetylase. Some of the corresponding carboxylic acids **6**, **8**, **10** and **13** are also inhibitors of histone deacetylase.

In Scheme 2 is shown a general route to hydroxamic acid derivatives containing an *N,N*-dimethylamino group (*e.g.* **25**), and the corresponding esters (*e.g.* **24**) from which they are made. Hydrolysis of esters such as **24** provides the corresponding carboxylic acids which may also be inhibitors of histone deacetylase. The hydroxamic acid derivatives such as **25** are especially noteworthy as inhibitors of histone deacetylase, and they possess close structural analogies to the trichostatin A, a potent inhibitor of histone deacetylase. Scheme 2 is illustrated with an *N,N*-dimethylamino group, but is intended to include a wide range of substituents attached to nitrogen, including but not limited to monoalkyl, dialkyl, alkyl together with aryl, diaryl, one or more

heterocyclic substituents and a wide variety of other substituents with unsaturated and/or heteroatom functionality. The synthetic route of Scheme 2 is intended to apply to additional substituents in either the *ortho-* or *para*-positions, or both. It is also intended to apply to more than one such amino (or similar *N*-substituent) placed at a combination of two or more of the *ortho-*, *meta-* or *para*-positions.

A principal feature of Scheme 2 is the reductive amination of a carbonyl compound (illustrated with formaldehyde, but applicable to a wide variety of carbonyl compounds) with the disulfide **18** followed by a cleavage using tri-*n*-butylphosphine (or other phosphine) to give *in situ* the thiol **20** which is reacted with an unsaturated bromo ester **17** to give the sulfide **21**. Reduction of **21** is conveniently achieved using di-isobutylaluminium hydride (DIBAL) to give the alcohol **22** which is treated with pyridine-sulfur trioxide complex to give the aldehyde **23** which need not be isolated and which is reacted *in situ* with a stabilised phosphorus ylid such as (triphenylphosphanylidene)-acetic acid ethyl ester to give **24** or a related sulfide that is reacted with hydroxylamine (or a salt or other derivative of hydroxylamine) to give the hydroxamic acid such as **25**. The route in Scheme 2 is particularly effective for the incorporation of amino and other nitrogen-containing substituents. The scope of the reductive amination is such that the aromatic amino group ($ArNH_2$) may be reacted first with one aldehyde or ketone under reductive conditions, and the resulting secondary amine then reacted with a second aldehyde or ketone (which may be the same as the first aldehdye or ketone or different from it), again under reductive conditions.

According to a fourth aspect of the invention, there is provided a pharmaceutical composition comprising a compound of general formula (I), and optionally a pharmaceutically acceptable adjuvant and/or diluent.

Therapeutic substances of the present invention may be used in the treatment of a human or non-human animal. The treatment may be prophylactic or may be in respect of an existing condition. For example, in the treatment of cancer. Thus the substances of the

present invention may be used in the manufacture of a medicament for the treatment of one or more of the above-mentioned diseases/disorders.

The medicament will usually be supplied as part of a sterile, pharmaceutical composition which will normally include a pharmaceutically acceptable carrier. This pharmaceutical composition may be in any suitable form, (depending upon the desired method of administering it to a patient).

It may be provided in unit dosage form, will generally be provided in a sealed container and may be provided as part of a kit. Such a kit would normally (although not necessarily) include instructions for use. It may include a plurality of said unit dosage forms.

The pharmaceutical composition may be adapted for administration by any appropriate route, for example by the oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual or transdermal), vaginal or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) route. Such compositions may be prepared by any method known in the art of pharmacy, for example by admixing the active ingredient with the carrier(s) or excipient(s) under sterile conditions.

Pharmaceutical compositions adapted for oral administration may be presented as discrete units such as capsules or tablets; as powders or granules; as solutions, syrups or suspensions (in aqueous or non-aqueous liquids; or as edible foams or whips; or as emulsions)

Suitable excipients for tablets or hard gelatine capsules include lactose, maize starch or derivatives thereof, stearic acid or salts thereof. Suitable excipients for use with soft gelatine capsules include for example vegetable oils, waxes, fats, semi-solid, or liquid polyols etc. For the preparation of solutions and syrups, excipients which may be used include for example water, polyols and sugars. For the preparation of suspensions oils (e.g. vegetable oils) may be used to provide oil-in-water or water in oil suspensions.

Pharmaceutical compositions adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. For example, the active ingredient may be delivered from the patch by iontophoresis as generally described in *Pharmaceutical Research*, 3(6):318 (1986).

Pharmaceutical compositions adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils. For infections of the eye or other external tissues, for example mouth and skin, the compositions are preferably applied as a topical ointment or cream. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an oil-in-water cream base or a water-in-oil base. Pharmaceutical compositions adapted for topical administration to the eye include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent. Pharmaceutical compositions adapted for topical administration in the mouth include lozenges, pastilles and mouth washes. Pharmaceutical compositions adapted for rectal administration may be presented as suppositories or enemas.

Pharmaceutical compositions adapted for nasal administration whereinthe carrier is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable compositions wherein the carrier is a liquid, for administration as a nasal spray or as nasal drops, include aqueous or oil solutions of the active ingredient.

Pharmaceutical compositions adapted for administration by inhalation include fine particle dusts or mists which may be generated by means of various types of metered dose pressurised aerosols, nebulizers or insufflators.

Pharmaceutical compositions adapted for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations.

Pharmaceutical compositions adapted for parenteral administration include aqueous and non-aqueous sterile injection solution which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation substantially isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. Excipients which may be used for injectable solutions include water, alcohols, polyols, glycerine and vegetable oils, for example. The compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carried, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

The pharmaceutical compositions may contain preserving agents, solubilising agents, stabilising agents, wetting agents, emulsifiers, sweeteners, colourants, odourants, salts (substances of the present invention may themselves be provided in the form of a pharmaceutically acceptable salt), buffers, coating agents or antioxidants. They may also contain therapeutically active agents in addition to the substance of the present invention.

Dosages of the substance of the present invention can vary between wide limits, depending upon the disease or disorder to be treated, the age and condition of the individual to be treated, etc. and a physician will ultimately determine appropriate dosages to be used.

This dosage may be repeated as often as appropriate. If side effects develop the amount and/or frequency of the dosage can be reduced, in accordance with normal clinical practice.

According to a fifth aspect of the invention there is provided a compound of general formula (I) for use in medicine.

Without wishing to be bound by theory, it is believed that the diseases in which the compounds of the present invention may find greatest application in medical treatment will be in the field of cancer. For example, in the treatment of cancerous tumour growths.

According to a sixth aspect of the present invention, there is provided a method of treatment of an individual suffering from a disease condition, the method comprising administering to the individual a therapeutically effective amount of a compound of general formula (I).

According to a seventh aspect of the present invention, there is provided a method of inhibition of histone deacetylase activity in an individual suffering from a disease condition, the method comprising administering to the individual a therapeutically effective amount of a compound of general formula (I).

The inhibition may be defined as any reduction in the activity of histone deacetylase activity in the individual. The reduction may be from an elevated level of activity to a normal level in the subject, or it may even be a reduction to below what would be considered as the normal activity in the subject.

According to a eighth aspect of the invention, there is provided the use of a compound of general formula (I) in the manufacture of a medicament for the treatment of cancer.

Preferred features for the second and subsequent aspects of the invention are as for the first aspect *mutatis mutandis*.

The invention will now be further described by way of reference to the following Examples which are provided for the purposes of illustration only and are not to be construed as being limiting on the invention.

Syntheses of preferred compounds:

Experimental Section

Starting materials were purchased from Avocado or Aldrich and used as supplied, unless otherwise stated.

(5-Acetylamino-1-carbamoyl-pentyl)-carbamic acid *tert*-butyl ester.

To a solution of triethylamine (0.8 mL, 5.8 mmol) in DMF (35 mL) was added *N*-acetyl-L-lysine (1.0 g, 5.3 mmol). After 15 minutes, di-*tert*-butyl dicarbonate (1.27 g, 5.8 mmol) was added to the slurry. After a further sixteen hours, the clear solution was concentrated under reduced pressure to give an oil. Water was added together with enough saturated aqueous sodium hydrogen carbonate to dissolve the oil. The basic solution was washed three times with diethyl ether to remove any unreacted di-*tert*-butyl dicarbonate. The aqueous solution was then cooled to 0 °C and acidified with concentrated hydrochloric acid (10 M). The oily mixture was extracted three times with ethyl acetate. The residual organic layer was then washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give the pure title compound as a white powder.

[(S)-5-Acetylamino-1-(4-methyl-2-oxo-2H-chromen-7-ylcarbamoyl)-pentyl]-carbamic acid-*tert*-butyl ester was prepared as described in: Hoffmann, K.; Brosch, G.; Loidl, P.; Jung, M. Pharmazie, **2000**, *55*, 601.

Hexa-2,4-dienoic acid methyl ester (3a).

To a solution of sorbic acid (1.12 g, 10 mmol) in dry methanol (50 mL) was added dropwise a solution of trimethylsilyl chloride (2M, 12 mL) in CH_2Cl_2. After stirring for 16 hours, the mixture was concentrated under reduced pressure to give a pale yellow oil that was purified by flash chromatography (9:1 60-80 °C petroleum ether: diethyl ether) to give the title compound (1.23 g, 99%) as a colourless oil.

6-Bromohexa-2,4-dienoic acid methyl ester (4a).

A mixture of methyl sorbate (1.26 g, 10 mmol) and *N*-bromosuccinimide (1.98 g, 11.0 mmol) in chlorobenzene was irradiated with a 250 W sunlamp so as to achieve a state of reflux for 4 hours. The (cooled) mixture was then evaporated under reduced pressure to give a brown oil which was purified by column chromatography (9:1 60-80 °C petroleum ether: diethyl ether) to give the title compound (1.33 g, 65 %) as a clear oil.

6-Phenylsulfanyl-hexa-2,4-dienoic acid (6a).

To a solution of 6-phenylsulfanyl-hexa-2,4-dienoic acid methyl ester (0.34 g, 1.45 mmol) in methanol (5 mL) were added aqueous sodium hydroxide (1M, 5 mL, 5 mmol) and distilled water (20 mL). The stirred mixture was heated at reflux for 1 hour, allowed to cool to 20 °C, and then concentrated under reduced pressure to about 10 mL. The aqueous solution was washed with diethyl ether (50 mL) and then poured onto a mixture of ethyl acetate (50 mL) and hydrochloric acid (2M, 10 mL) at 0 °C. The mixture was shaken, the organic layer was separated and washed successively with saturated aqueous sodium hydrogen carbonate (25 mL), distilled water (25 mL), and brine (25 mL), then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give a pale yellow solid that was recrystallised from diethyl ether/hexanes to give the title compound (0.30 g, 93 %) as a white solid, mp 108-110 °C.

6-(4-Chlorophenylsulfanyl)-hexa-2,4-dienoic acid methyl ester (6b).

To a solution of 6-bromohexa-2,4-dienoic acid methyl ester (1.03 g, 5 mmol), triethylamine (1.4 mL, 10 mmol) and *tert*-butylammonium iodide (92 mg, 0.25 mmol) in freshly distilled THF (25 mL) was added 4-chlorothiophenol under an atmosphere of nitrogen. The mixture was stirred at reflux for 2 hours after which it was concentrated under reduced pressure. The oil was dissolved in ethyl acetate (100 mL) and then washed successively with saturated aqueous sodium hydrogen carbonate (50 mL), distilled water (50 mL) and brine (50 mL). The organic layer was then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give an oil that was purified by flash chromatography (1:9 diethyl ether: 40-60 °C petroleum ether). The resulting yellow solid was recrystallised from diethyl ether/40-60 °C

petroleum ether to give the title compound (0.85 g, 63%) as a white solid, mp 75-77 °C.

6-Phenylsulfanyl-hexa-2,4-dienoic acid methyl ester (6c).

To a solution of 6-bromohexa-2,4-dienoic acid methyl ester (0.57 g, 2.8 mmol) and thiophenol (0.28 mL, 2.8 mmol) was added triethylamine (0.43 mL, 3.1 mmol), dropwise, under an atmosphere of nitrogen. The mixture was stirred at 20 °C for one hour. The slurry was then filtered and the filtrate was washed with aqueous sodium hydroxide (1 M) then with brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was subjected to column chromatography (9:1 60-80 °C petroleum ether: diethyl ether) to give the title compound (0.93 g, 88%) as a clear oil.

6-(4-Dimethylamino-phenylsulfanyl)-hexa-2,4-dienoic acid methyl ester (6d).

To a solution of 6-bromo-hexa-2,4-dienoic acid methyl ester (1.15 g, 5.6 mmol) and 4-dimethylamino-benzenethiol (0.86 g, 5.6 mmol) in tetrahydrofuran (30 mL) was added dropwise triethylamine (1.6 mL, 11.4 mmol) under an atmosphere of argon. The mixture was stirred at 20 °C for 30 minutes, then filtered. The filtrate was concentrated under reduced to pressure and the residue was dissolved in ethyl acetate (100 mL). This solution was washed with saturated aqueous sodium hydrogen carbonate (50 mL), demineralised water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give a yellow oil that was purified by column chromatography (9:1 to 7:3 60-80 °C petroleum ether: diethyl ether, all eluant containing 1% triethylamine by volume). The solid was recrystallised from diethyl ether/petroleum ether to give the title compound (1.0 g, 64%) as a white solid, mp 68-70 °C.

6-(4-Methoxy-phenylsulfanyl)-hexa-2,4-dienoic acid methyl ester (6e).

To a solution of 6-bromo-hexa-2,4-dienoic acid methyl ester (1.50 g, 7.3 mmol) and 4-methoxythiophenol (0.93 mL, 7.3 mmol) in tetrahydrofuran (40 mL) was added dropwise triethylamine (1.1 mL, 8.0 mmol) under an atmosphere of argon. The mixture was stirred at 20 °C for one hour, then filtered. The filtrate was concentrated

under reduced to pressure and the residue was dissolved in ethyl acetate (50 mL). This solution was washed with saturated aqueous sodium hydrogen carbonate (25 mL), demineralised water (25 mL) and brine (25 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give a yellow oil that was purified by column chromatography ((9:1 to 8:2 40-60 °C petroleum ether: diethyl ether) to give the title compound as a colourless oil (1.6 g, 86%).

6-(4-Chlorophenylsulfanyl)-hexa-2,4-dienoic acid hydroxyamide (7b).

To a solution of the 6-(4-chlorophenylsulfanyl)-hexa-2,4-dienoic acid methyl ester (0.44 g, 1.64 mmol) in distilled THF (9.0 mL) containing 50% aqueous hydroxylamine (1.0 ml, 15.2 mmol) was added at 0 °C a solution of potassium hydroxide in methanol (1M, 2.6 mL, 2.6 mmol) over a period of 30 minutes. After stirring at 0 °C for 1 h, distilled water (9.0 mL) was added and the mixture was made neutral by dropwise addition of concentrated hydrochloric acid (10 M) at 0 °C. The aqueous solution was extracted with ethyl acetate (3 x 30 mL) and the combined extracts were dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was recrystallised from acetone to give the title compound (0.21 g, 48%) as a pale brown powder, mp 120-122 °C (decomp).

6-(4-Dimethylamino-phenylsulfanyl)-hexa-2,4-dienoic acid hydroxyamide (7c).

To a solution of 6-(4-dimethylamino-phenylsulfanyl)-hexa-2,4-dienoic acid methyl ester (0.556 g, 2.0 mmol) in distilled THF (10 mL) containing aqueous hydroxylamine (50%, 1.21 mL, 18.4 mmol) was added at 0 °C a solution of potassium hydroxide in methanol (1M, 2.8 mL, 2.8 mmol) over a period of 30 minutes. After stirring the mixture at 0 °C for an additional hour, distilled water (10 mL) was added and the mixture was made neutral by dropwise addition of concentrated hydrochloric acid (10 M) at 0 °C. The solution was then extracted with ethyl acetate (2 x 50 mL), and the combined extracts were dried over anhydrous $MgSO_4$, and evaporated to dryness. The solid residue was recrystallised from ethyl acetate to give the title compound (0.24 g, 43%) as a white solid.

6-Benzenesulfinyl-hexa-2,4-dienoic acid methyl ester (8a).

To a solution of 6-phenylsulfanyl-hexa-2,4-dienoic acid methyl ester (0.336 g, 1.43 mmol) in methanol (17 mL) was added dropwise at 0 °C a solution of sodium metaperiodate (0.37 g, 1.72 mmol) in distilled water (6 mL). The mixture was allowed to warm to 20 °C and then heated at reflux for 5 hours. The solution was concentrated under reduced pressure to give an oil that was dissolved in ethyl acetate (20 mL). The organic layer was washed with saturated aqueous sodium hydrogen carbonate (10 mL), distilled water (10 mL) and then brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give an oil that was purified by flash chromatography (1:2 to 1:1 to 2:1 ethyl acetate: 60-80 °C petroleum ether). The resulting powder was recrystallised from diethyl ether to give the title compound (0.29 g, 81%) as a white solid, mp 82-84 °C.

6-(4-Chloro-benzenesulfinyl)-hexa-2,4-dienoic acid methyl ester (8b).

To a solution of 6-(4-chlorophenylsulfanyl)-hexa-2,4-dienoic acid methyl ester (0.396 g, 1.47 mmol) in methanol (20 mL) was added dropwise at 0 °C a solution of sodium metaperiodate (0.37 g, 1.72 mmol) in distilled water (10 mL). The mixture was allowed to warm to 20 °C, then heated at reflux for 5 hours. The solution was concentrated under reduced pressure to give an oil that was dissolved in ethyl acetate (20 mL). The organic layer was washed with saturated aqueous sodium hydrogen carbonate (10 mL), distilled water (10 mL), then with brine. The organic layer was then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give an oil that was purified by flash chromatography (2:8 ethyl acetate: 40-60 °C petroleum ether). The resulting powder was recrystallised from ethylacetate/40-60 °C petroleum ether to give the title compound (0.32 g, 77%) as a white solid.

6-(4-Methoxybenzenesulfinyl)-hexa-2,4-dienoic acid methyl ester (8c).

To a solution of 6-(4-methoxybenzenesulfanyl)-hexa-2,4-dienoic acid methyl ester (1.0 g, 3.8 mmol) in methanol (8 mL) was added at 0 °C a solution of sodium metaperiodate (0.856 g, 4.0 mmol) in water (8 mL). The mixture was stirred at reflux for 1 h, allowed to cool to 20 °C then extracted with ethyl acetate (3 x 50 mL). The combined extracts were washed with saturated aqueous sodium hydrogen carbonate (25 mL), distilled water (25 mL) and brine (25 mL), dried over anhydrous $MgSO_4$,

and evaporated to dryness. The residue was purified by column chromatography (1:1 to 8:2 ethyl acetate: 40-60 °C petroleum ether) to give the title compound (0.92 g, 87 %) as a pale yellow oil.

6-Benzenesulfinyl-hexa-2,4-dienoic acid (8d).

To a solution of 6-benzenesulfinyl-hexa-2,4-dienoic acid methyl ester (0.243 g, 0.97 mmol) in methanol (2.0 mL) were added a solution of aqueous sodium hydroxide (1M. 4.0 mL, 4 mmol) and distilled water (10 mL). After stirring at reflux for one hour, the mixture was allowed to cool to 20 °C and was concentrated under reduced pressure. The solution was washed with diethyl ether (25 mL) and then poured onto a mixture of ethyl acetate (20 mL) and hydrochloric acid (2M, 5 mL) at 0 °C. The mixture was shaken, and the organic layer separated and washed successively with saturated aqueous sodium hydrogen carbonate (10 mL), distilled water (10 mL), brine (10 mL), then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give a pale yellow solid that was recrystallised from ether/hexanes to give the title compound (0.204 g, 89 %) as a white solid.

6-(4-Chlorobenzenesulfinyl)-hexa-2,4-dienoic acid hydroxyamide (9a).

To a solution of the 6-(4-chlorobenzenesulfinyl)-hexa-2,4-dienoic acid methyl ester (0.80 g, 2.79 mmol) in distilled THF (15 mL) containing an aqueous solution of hydroxylamine (50%, 1.7 ml, 25.8 mmol) was added at 0 °C a solution of potassium hydroxide in methanol (1M, 4.5 ml, 4.5 mmol) over a period of 30 minutes. After stirring at 0 °C for 1 hour, distilled water (15 mL) was added and the mixture was made neutral by dropwise addition of concentrated hydrochloric acid (10 M) at 0 °C. The aqueous solution was extracted with ethyl acetate (3 x 50 mL) and the combined extracts were dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was recrystallised from acetone to give the title compound (0.45 g, 56%) as a pale brown powder, mp 159 °C (decomp).

6-(4-Methoxybenzenesulfinyl)-hexa-2,4-dienoic acid hydroxyamide (9b).

To a solution of 6-(4-methoxybenzenesulfinyl)-hexa-2,4-dienoic acid methyl ester (0.32 g, 1.14 mmol) in distilled THF (6 mL) containing an aqueous solution of hydroxylamine (50%, 0.7 ml, 10.6 mmol) was added at 0 °C a solution of potassium

hydroxide in methanol (1M, 1.8 ml, 1.8 mmol) over a period of 30 minutes. After stirring at 0 °C for 1 hour, distilled water (6 mL) was added and the mixture was made neutral by dropwise addition of concentrated hydrochloric acid (10 M) at 0 °C. The aqueous solution was extracted with ethyl acetate (3 x 20 mL) and the combined extracts were dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was recrystallised from acetone to give the title compound (0.11 g, 34%) as a pale brown powder, mp 145-147 °C (decomp).

6-Benzenesulfonyl-hexa-2,4-dienoic acid (10a).

To a solution of the 6-benzenesulfonyl-hexa-2,4-dienoic acid methyl ester (0.70 g, 2.60 mmol) in methanol (8 mL) were added a aqueous sodium hydroxide 1M, 6.0 mL, 6 mmol) and distilled water (20 mL). After stirring at reflux for 1 hour, the mixture was allowed to cool to 20 °C and was concentrated under reduced pressure. The aqueous solution was washed with diethyl ether (50 mL) and then poured onto a mixture of ethyl acetate (50 mL) and hydrochloric acid (2M, 5 mL) at 0 °C. The organic layer was washed successively with saturated aqueous sodium hydrogen carbonate (20 mL), distilled water (20 mL), and brine (20 mL), then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give a pale yellow solid that was recrystallised from ethyl acetate to give the title compound (0.58 g, 87%) as a white solid, mp 148-150 °C.

6-Benzenesulfonyl-hexa-2,4-dienoic acid methyl ester (10b).

A mixture of 6-bromohexa-2,4-dienoic acid methyl ester (0.41 g, 2.0 mmol), sodium benzene sulfinate (0.33 g, 2.0 mmol) and tetra-*n*-butylammonium iodide (37 mg, 0.1 mmol) was heated at reflux in dry THF (10 mL) under an atmosphere of nitrogen for 2 hours. The resulting slurry was allowed to cool to 20 °C and filtered. The filtrate was concentrated under reduced pressure to give an oil that was dissolved in ethyl acetate (20 mL). This solution was washed successively with saturated aqueous sodium hydrogen carbonate (10 mL), distilled water (10 mL), then with brine (10 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give an oil that was purified by flash chromatography (7:3

to 1:1 to 1:1 60-80 °C petroleum ether: diethyl ether) to give a solid that was recrystallised to give the title compound (0.39 g, 73%) as white crystals, mp 105 °C.

6-Benzenesulfonyl-hexa-2,4-dienoic acid hydroxyamide (11a).

To a solution of 6-benzenesulfonyl-hexa-2,4-dienoic acid (0.292 g, 1.15 mmol) in dry dichloromethane (5 mL) was added dropwise oxalyl chloride, at 0 °C under an atmosphere of argon. One drop on dimethylformamide was then added. After stirring at 20 °C for one hour, a solution of aqueous hydroxylamine (50%, 0.17 mL, 2.5 mmol) in tetrahydrofuran (5 mL) was added. The mixture was stirred for an additional hour and concentrated under reduced pressure to give a yellow foam that was purified by column chromatography (8:2 ethyl acetate: 40-60 °C petroleum to 100% ethyl acetate). The title compound (91 mg, 31%) was obtained as a white foam.

6-(Naphthalen-2-ylsulfanyl)-hexa-2,4-dienoic acid methyl ester (13b).

To a solution of 6-bromo-hexa-2,4-dienoic acid methyl ester (1.57 g, 7.66 mmol) and 2-thionaphthol (1.35 g, 8.42 mmol) in diethyl ether (40 mL) was added dropwise triethylamine (1.40 mL, 10.0 mmol) under an atmosphere of argon. The mixture was stirred for one hour 20 °C and filtered. The filtrate was washed saturated aqueous sodium hydrogen carbonate (25 mL), demineralised water (25 mL) and brine (25 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give a yellow oil that was purified by column chromatography (9:1 to 8:2 40-60 °C petroleum ether: diethyl ether). Recrystallisation from diethyl ether/ petroleum ether afforded the title compound (1.9 g, 88%) as a white solid, mp 73-75 °C.

6-(Naphthalen-2-ylsulfanyl)-hexa-2,4-dienoic acid hydroxyamide (14a).

To a solution of 6-(naphthalen-2-ylsulfanyl)-hexa-2,4-dienoic acid methyl ester (0.505 g, 1.78 mmol) in distilled THF (10 mL) containing 50% aqueous hydroxylamine (1.08 mL, 16.4 mmol) was added at 0 °C a solution of potassium hydroxide in methanol (1M, 2.5 ml, 2.5 mmol) over a period of 30 minutes. After stirring the mixture at 0 °C for an additional hour, distilled water (10 mL) was added and the mixture was made neutral by dropwise addition of concentrated hydrochloric

acid (10 M) at 0 °C. The solution was extracted with ethyl acetate (2 x 50 mL), and the combined extracts were dried over anhydrous $MgSO_4$, and evaporated to dryness. The solid residue was recrystallised from ethyl acetate to give the title compound (0.315 g, 62%) as a white solid.

2-Methylpent-2-enoic acid methyl ester (16b).

A mixture of 2-methylpent-2-enoic acid (5.0 g, 44 mmol) and sulfuric acid (0.5 mL) in methanol (250 mL) was heated at reflux for 16 hours. The solution was allowed to cool down to 20 °C and concentrated under reduced pressure. The residue was dissolved in ethyl acetate (100 mL) and washed with saturated aqueous sodium hydrogen carbonate (2 x 50 mL), distilled water (50 mL), then with brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give the title compound (5.1 g, 91%) as a colourless oil.

4-Bromo-2-methylpent-2-enoic acid methyl ester (17b).

A solution of 2-methylpent-2-enoic acid methyl ester (1.20 g, 9.37 mmol) and *N*-bromosuccinimide (1.83 g, 10.2 mmol) in dry carbon tetrachloride (15 mL) irradiated with a 250 W sunlamp so as to achieve a state of reflux for 2.5 hours. After cooling, the succinimide was filtered off, the carbon tetrachloride was evaporated, and the product was distilled over a short path under reduced pressure to give the title compound (1.60 g, 82 %) as a colourless oil.

4-(*N*,*N*-Dimethylamino)phenyl disulfide (19).

To a stirred solution of 4-aminophenyl disulfide (2.50 g, 10 mmol) in methanol (60 mL) containing 37% aqueous formaldehyde (25 mL, 62.5 mmol) at 20 °C was added a solution of sodium cyanoborohydride (2.60 g, 40 mmol) and zinc chloride (2.74 g, 20 mmol) in methanol (100 mL). After stirring at 20 °C for 2 h, the solution was dissolved in NaOH (0.1 M, 100 mL) and most of the methanol was evaporated under reduced pressure. The aqueous solution was extracted with ethyl acetate (3 x 200 mL) and the combined extracts were washed with water then with brine, dried over

anhydrous $MgSO_4$, and evaporated to dryness. The residue was recrystallised from methanol to a give the title compound (2.48 g, 81%) as a yellow solid.

4-Dimethylamino-benzenethiol (20).

To a stirred solution of 4-(*N,N*-dimethylamino)phenyl disulfide (0.855 g, 2.81 mmol) in a mixture of dioxane (10 mL) and demineralised water (2.5 mL) at 20 °C was added tri-*n*-butylphosphine (0.75 mL, 2.89 mmol). After stirring at 20 °C for 30 minutes, the mixture was concentrated under reduced pressure. The residue was dried by co-evaporation with toluene (3 x 10 mL) to afford a clear oil containing the title compound (2.81 mmol) which was used without any further purification.

4-(4-Dimethylaminophenylsulfanyl)-2-methylpent-2-enoic acid methyl ester (21b).

To a solution of 4-bromo-2-methylpent-2-enoic acid methyl ester (0.414 g, 2.0 mmol) in dry benzene (4 mL) under an atmosphere of argon was added a solution of triethylamine (0.28 mL, 2.0 mmol) and 4-dimethylamino-benzenethiol (0.306 g, 2.0 mmol) in dry benzene (2 mL). The mixture was stirred at 20 °C for 2 hours (TLC monitoring). The solvent was then removed under reduced pressure to give a yellow oil that was purified by column chromatography (20:79:1 diethyl ether: 60-80 °C petroleum ether: triethylamine) to give the title compound (0.53 g, 95 %) as a colourless oil.

4-(4-Dimethylaminophenylsulfanyl)-2-methylpent-2-en-1-ol (22b).

To a solution of 4-(4-dimethylaminophenylsulfanyl)-2-methylpent-2-enoic acid methyl ester (0.279 g, 1.0 mmol) in dry THF (4 mL) at 0 °C was added dropwise a solution of di-isobutylaluminium hydride in toluene (1M, 3.0 mL, 3.0 mmol), under an atmosphere of argon. After stirring at 0 °C for one hour (TLC monitoring), the mixture was quenched by addition of methanol (0.5 mL: CAUTION), then water (0.3 mL), 5% aqueous sodium hydroxide solution (0.3 mL) and lastly 30% aqueous sodium potassium tartrate (4 mL). The mixture was stirred for 2 hours at 20 °C, then extracted with diethyl ether (3 x 10 mL). The combined organic layers were dried

over anhydrous $MgSO_4$, filtered and evaporated to give the title compound (0.22 g, 88%) as a colourless oil.

6-(4-Dimethylaminophenylsulfanyl)-4-methylhepta-2,4-dienoic acid ethyl ester (24c).

To a solution of 4-(4-dimethylamino-phenylsulfanyl)-2-methylpent-2-en-1-ol (0.739 g, 2.94 mmol) and triethylamine (3.8 mL, 27.1 mmol) in dimethyl sulfoxide (10 mL) was added sulfur trioxide-pyridine complex 50% (2.76 mg, 8.68 mmol) in dimethyl sulfoxide (10 mL). The mixture was stirred at 20 °C for 10 minutes (TLC monitoring) and ice-water (50 mL) was added. The mixture was extracted with diethyl ether (50 mL). The ethereal layer was washed with water (3 x 25 mL) then with brine (25 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The resulting yellow oil was dissolved in dichloromethane (15 mL) and (triphenylphosphanylidene)-acetic acid ethyl ester was added in one portion. The mixture was gently heated at reflux for 24 h under an atmosphere of argon. The solvent was removed under reduced pressure and the crude material was purified by column chromatography (10:89:1 diethyl ether: 40-60 °C petroleum ether: triethylamine) to give the title compound (0.71 g, 76%) as a colourless oil.

6-(4-Dimethylamino-phenylsulfanyl)-4-methylhepta-2,4-dienoic acid hydroxyamide (25c).

To a solution of 6-(4-dimethylaminophenylsulfanyl)-4-methylhepta-2,4-dienoic acid ethyl ester (0.576 g, 1.80 mmol) in dry THF (10 mL) was added at 0 °C an aqueous solution of hydroxylamine (50%, 1.1 mL, 16.6 mmol). To this mixture was added a solution of potassium hydroxide in methanol (1M, 2.9 mL, 2.9 mmol) over a period of 40 minutes at 0 °C. The mixture was stirred at 20 °C for 16 hours, and water (10 mL) was then added. The solution was acidified to pH 5 by addition of concentrated hydrochloric acid (10 M, CAUTION), then extracted with ethyl acetate (50 mL). The organic layer was washed with brine (25 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The yellow oil was dissolved in a mixture of 1:1 diethyl ether: methanol (20 mL) and 20 drops of concentrated hydrochloric (10 M) were added. The crude mixture was evaporated to dryness to give an oil that was

triturated with a mixture of diethyl ether and ethanol to give the title compound (0.34 g, 52%) as an off-white solid, mp 177-178 °C (decomp).

Activity assay:

The activity of the compounds as inhibitors of histone deacetylase was investigated using a modified assay based on a rapid *in vitro* HDAC activity assay (based on the deacetylation of an Ω-acetylated lysine (MAL)) and described by Hoffman *et al* in *Nucl. Acids. Res.* 27 2057-2058 (1999).

The HDAC substrate N-(-4-methyl-7-coumarinyl)-N-α-(*tert*-butyloxy-carbonyl)-N-Ω abbreviated as MAL was synthesised as described in Hoffman *et al* (1999). HDAC inhibitors and substrate (MAL) were made up in Hepes buffer (50mM, pH 7.4). Purified HDAC (100µL), inhibitor or Hepes buffer (100µl), substrate (MAL, 100µL 5µg/mL) and assay buffer (100µL, tris-HCl (10mM), NaCl (10mM), $MgCl_2$ (15mM), EGTA (0.1mM), 10% (v/v) glycerol, and mercaptoethanol (0.007%)) were incubated at 37°C. The reaction was terminated with 100µl acetonitrile, and MAL and the deacetylated produce (ML) were determined in the supernatant.

The assay devised by the inventors uses intact cells with a single time-point reaction. $1x10^6$ CEM cells in 1ml medium were exposed to inhibitors at 6 concentrations for 60 minutes, after which MAL at 20µg/ml (5µg/mL final concentration) was added for a further 30 minutes, all at 37°C. Cells were then rapidly washed at 4°C, lysed by sonication, the reaction stopped with acetonitrile, and MAL and the deacetylated product determined in the supernatant by rapid HPLC.

For assays with partially purified rat liver HDAC enzyme, substrate (5µg/ml MAL) and inhibitor at 6 concentrations were incubated at 37°C for 60 minutes, after which the reaction was stopped and MAL and ML determined in the supernatant.

Chromatographic separation of MAL and ML was carried out using a 15cm Apex ODS 5µM column with acetonitrile/distilled water (40:60), 2% trifluoracetic acid

(TFA) v/v mobile phase at a flow rate of 1.2 ml/minute. MAL and ML were quantified by fluorescence detection at excitation/emission wavelengths of 330/395nm.

The activity of each inhibitor was assessed at a minimum of 5 non-zero concentrations. MAL and ML peak heights were used to derive the percentage MAL in the mixture as the ration of MAL: MAL+ML. The percentage MAL in the absence of inhibitor (typically 22-25%) was taken as 100% HDAC activity, and the percentage HDAC activity at higher concentrations derived from (100%-%MALdrug/100-%MALnodrug x 100). These data (minimum of n=4 at each concentration for each inhibitor) were fitted to a sigmoidal EMAX model (Graphpad Prism ver 2.01) to derive the IC_{50} concentration for each inhibitor.

These assays have been used to investigate the activity of known and novel HDAC inhibitors. The requirement for a hydroxamic acid moiety for potent HDAC inhibition was confirmed in the activity of sodium phenylbutyrate (NaPB)(HDAC activity IC_{50}; rat liver HDAC 153 ± 51 μM, CEM cells 5572 ± 2001 μM) and its hydroxamic acid (NaPBHA) derivative (rat liver HDAC 6.2 ± 2.0 μM, CEM cells 158 ± 99 μM).

IC50 values for percentage viability (3-day exposure) in CEM cells for NaPB (7800 ± 2100 μM) and NaPBHA (138 ± 28 μM) agreed much more closely with the whole cell HDAC activity values than with rat liver enzyme values, suggesting the whole cell assay gives a much better indication of potential biological activity.

Table 1

Inhibitor	HDAC inhibitory activity (IC_{50}) μM Liver preparation	HDAC inhibitory activity (IC_{50}) μM CEM cells	% viability (IC_{50}) μM
sodium phenylbutyrate (NaPB)	153 ± 51	5572 ± 2001	7800 ± 2100
sodium phenyl-butyrohydroxamic acid (NaPBHA)	6.2 ± 2.0	158 ± 99	138 ± 28

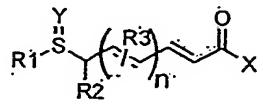
Inhibitor	HDAC inhibitory activity (IC_{50}) μM Liver preparation	HDAC inhibitory activity (IC_{50}) μM CEM cells	% viability (IC_{50}) μM
trichostatin	0.016 ± 0.005	0.019 ± 0.003	0.082 ± 0.011
hexa-2,4-dienic acid hydroxamide	0.8 ± 0.7	47 ± 17	ND
6-benzenesulfonyl-hexa-2,4-dienoic acid hydroxamide (CM4)	0.8 ± 0.3	3.3 ± 1.3	ND
6-(-4-chloro-benzenesulfinyl)-hexa-2,4-dienoic acid hydroxamide (CM5)	0.4 ± 0.1	1.2 ± 0.4	ND

Investigation of a number of simple, unsaturated hydroxamic acids found the straight chain hexa-2,4-dienic acid hydroxamide to have good HDAC inhibitory activity (rat liver HDAC IC_{50} 0.8 ± 0.7 μM), but the HDAC inhibitory activity was substantially lower in intact cells (IC_{50} 47 ± 17 μM).

However, increased activity resulted from the novel addition of a phenyl or chlorophenyl group to the hexa-2,4-dienic acid hydroxamide backbone via a sulphur atom as represented by compounds of the present invention 6-benzenesulfonyl-hexa-2,4-dienoic acid hydroxamide (CM4) and 6-(-4-chloro-benzenesulfinyl)-hexa-2,4-dienoic acid hydroxamide (CM5).

CLAIMS

1. A compound of general formula (1):



1

in which:

R^1 is (C_6 or C_{10}) aryl, (C_6 or C_{10}) arylalkyl, (C_6 or C_{10}) heteroaryl, (C_3-C_8) heterocycloalkenyl, (C_5-C_8) cycloalkene ring, (C_5-C_8) cycloalkyl, (C_5-C_8) heterocycloalkyl or a combination thereof to form a linked or fused ring system, the cyclic moiety being optionally substituted with (C_1-C_{10}) alkyl, (C_1-C_{10}) alkenyl, (C_1-C_{10}) alkynyl, (C_1-C_{10}) alkoxy, (C_1-C_{10}) thioalkoxy, hydroxyl, hydroxyl, (C_1-C_{10}) hydroxylalkyl, halo, (C_1-C_{10}) haloalkyl, amino, amido, (C_1-C_{10}) alkylcarbonyloxy, (C_1-C_{10}) alkoxycarbonyl, (C_1-C_{10}) alkylcarbonyl, (C_1-C_{10}) alkylthiocarbonyl, (C_1-C_{10}) alkylsulfonylamino, aminosulfonyl, (C_1-C_{10}) alkylsulfinyl, or (C_1-C_{10}) alkylsulfonyl,

R^2 and R^3 are each independently hydrogen, (C_1-C_6) alkyl, substituted (C_1-C_6) alkyl, or unsaturated (C_1-C_6) comprising one or more C=C bond or C≡C bond, (C_6 or C_{10}) aryl or (C_6 or C_{10}) heteroaryl, or a combination thereof to form a linked or fused ring system, or (C_1-C_{10}) alkyl, (C_1-C_{10}) alkenyl, (C_1-C_{10}) alkynyl, (C_1-C_{10}) alkoxy, (C_1-C_{10}) thioalkoxy, hydroxyl, hydroxyl, (C_1-C_{10}) hydroxylalkyl, halo, (C_1-C_{10}) haloalkyl, cyano, nitro, amino, amido, (C_1-C_{10}) alkylcarbonyloxy, (C_1-C_{10}) alkoxycarbonyl, (C_1-C_{10}) alkylcarbonyl, (C_1-C_{10}) alkylthiocarbonyl, (C_1-C_{10}) alkylsulfonylamino, aminosulfonyl, (C_1-C_{10}) alkylsulfinyl, or (C_1-C_{10}) alkylsulfonyl, or a saturated C3-C12 hydrocarbon chain or an unsaturated C_3-C_{12} hydrocarbon chain optionally interrupted by O, S, NR, CO, C(NR), N(R)SO_2, SO_2N(R), N(R)C(O)O, OC(O)N(R), N(R)C(O)N(R), OC(O), C(O)O, OSO_2, SO_2O, or OC(O)O, where R may be independently hydrogen, (C_1-C_{10}) alkyl, (C_1-C_{10}) alkenyl, (C_1-C_{10}) alkynyl, (C_1-C_{10}) alkoxy, (C_1-C_{10}) hydroxylalkyl, hydroxyl, (C_1-C_{10}) halolalkyl, where each of the saturated or unsaturated hydrocarbon chains may be optionally substituted with (C_1-C_{10}) alkyl, (C_1-C_{10}) alkenyl, (C_1-C_{10}) alkynyl, (C_1-C_{10}) alkoxy, hydroxyl, hydroxyl,

(C_1-C_{10}) hydroxylalkyl, halo, (C_1-C_{10}) haloalkyl, amino, (C_1-C_{10}) alkylcarbonyloxy, (C_1-C_{10}) alkoxycarbonyl, (C_1-C_{10}) alkylcarbonyl, (C_1-C_{10}) alkylsulfonylamino, aminosulfonyl, or (C_1-C_{10}) alkylsulfonyl,

or R^2 and R^3 optionally form a fused ring system together,

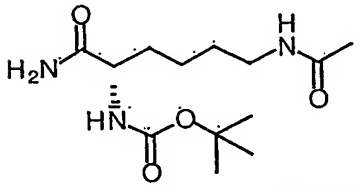
n is equal to 0, 1 or 2,

X is hydroxyl (-OH), hydroxamate (-NHOH), $NHOR^4$, NR^5OR^4, NR^5NHR^6,

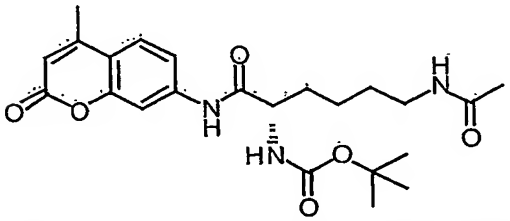
where R^4, R^5 or R^6 are each independently be hydrogen, C_1-C_6 alkyl or substituted C_1-C_6 alkyl; and

Y is 0, 1 or 2 oxygen atoms, or NR^7 where R^7 is OH, OR^8 or a carbon atom, where R^8 is C_1-C_6 alkyl or substituted C_1-C_6 alkyl.

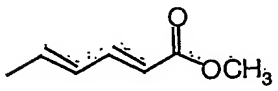
2. A compound as claimed in claim 1 which is:



(5-Acetylamino-1-carbamoyl-pentyl)-carbamic acid *tert*-butyl ester

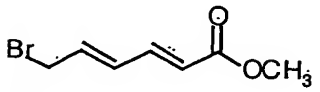


[(S)-5-Acetylamino-1-(4-methyl-2-oxo-2H-chromen-7-ylcarbamoyl)-pentyl]-carbamic acid-*tert*-butyl ester



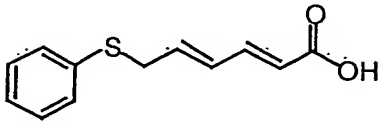
3a

Hexa-2,4-dienoic acid methyl ester



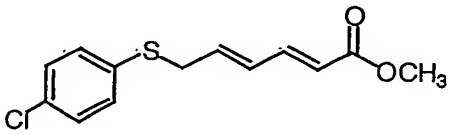
4a

6-Bromo-hexa-2,4-dienoic acid methyl ester



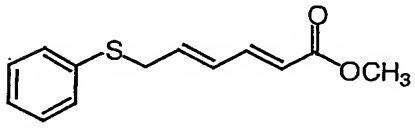
6a

6-Phenylsulfanyl-hexa-2,4-dienoic acid



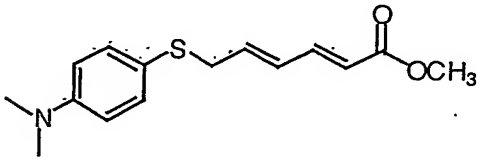
6b

6-(4-Chloro-phenylsulfanyl)-hexa-2,4-dienoic acid methyl ester

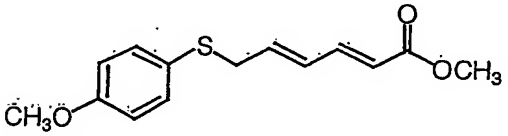


6c

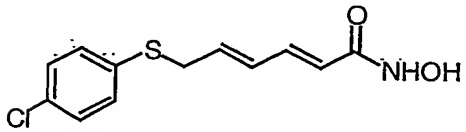
6-Phenylsulfanyl-hexa-2,4-dienoic acid methyl ester



6-(4-Dimethylamino-phenylsulfanyl)-hexa-2,4-dienoic acid methyl ester **6d**

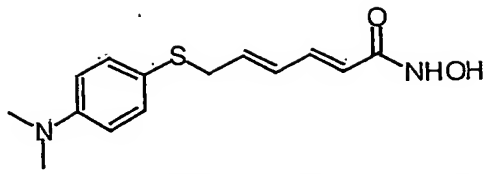


6-(4-Methoxy-phenylsulfanyl)-hexa-2,4-dienoic acid methyl ester **6e**

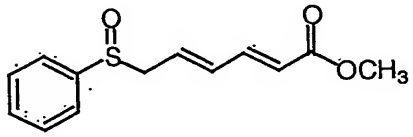


7b

6-(4-Chloro-phenylsulfanyl)-hexa-2,4-dienoic acid hydroxyamide

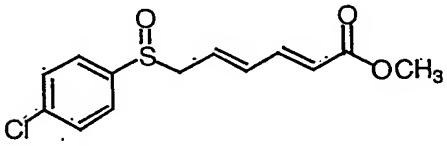


6-(4-Dimethylamino-phenylsulfanyl)-hexa-2,4-dienoic acid hydroxyamide **7c**



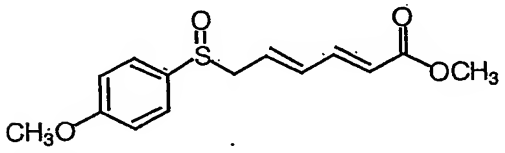
8a

6-Phenylsulfinyl-hexa-2,4-dienoic acid methyl ester



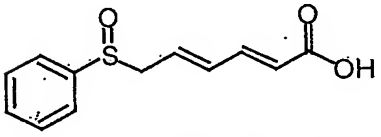
8b

6-(4-Chloro-benzenesulfinyl)-hexa-2,4-dienoic acid methyl ester

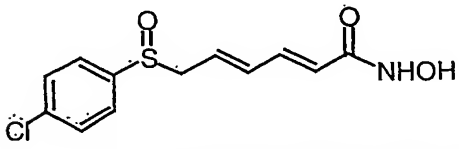


8c

6-(4-Methoxy-benzenesulfinyl)-hexa-2,4-dienoic acid methyl ester

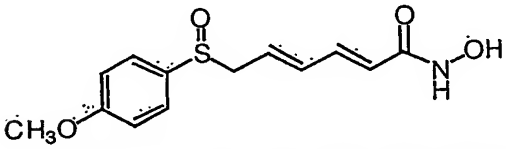


6-Benzenesulfinyl-hexa-2,4-dienoic acid **8d**



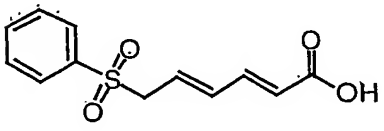
9a

6-(4-Chloro-benzenesulfinyl)-hexa-2,4-dienoic acid hydroxyamide



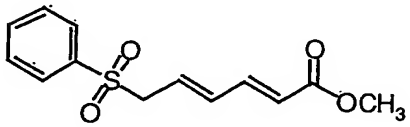
9b

6-(4-Methoxy-benzenesulfinyl)-hexa-2,4-dienoic acid hydroxyamide

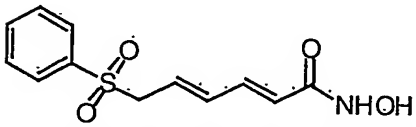


10a

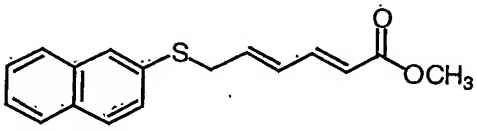
6-Benzenesulfonyl-hexa-2,4-dienoic acid



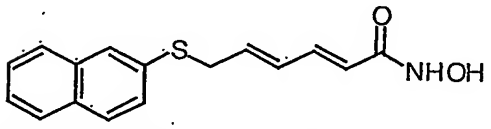
6-Benzenesulfonyl-hexa-2,4-dienoic acid methyl ester **10b**



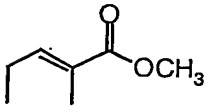
6-Benzenesulfonyl-hexa-2,4-dienoic acid hydroxyamide **11a**



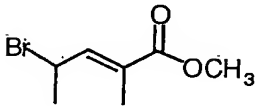
6-(Naphthalen-2-ylsulfanyl)-hexa-2,4-dienoic acid methyl ester **13b**



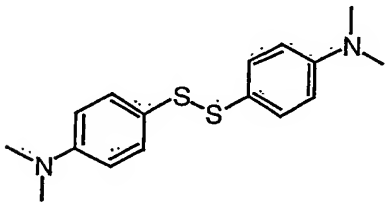
6-(Naphthalen-2-ylsulfanyl)-hexa-2,4-dienoic acid hydroxyamide **14a**



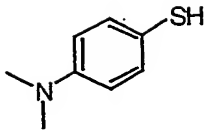
2-Methyl-pent-2-enoic acid methyl ester **16b**



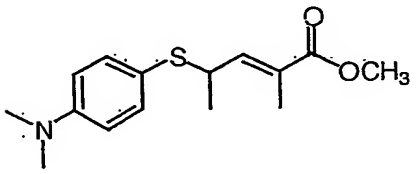
4-Bromo-2-methyl-pent-2-enoic acid methyl ester **17b**



4-(N,N-dimethylamino)phenyl disulfide **19**

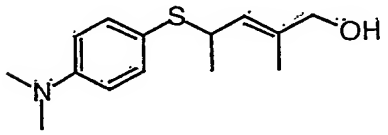


4-Dimethylamino-benzenethiol **20**



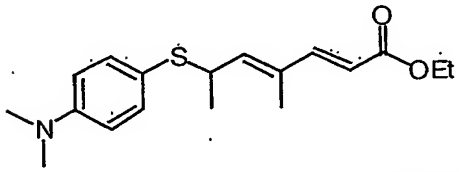
21b

4-(4-Dimethylamino-phenylsulfanyl)-2-methyl-pent-2-enoic acid methyl ester



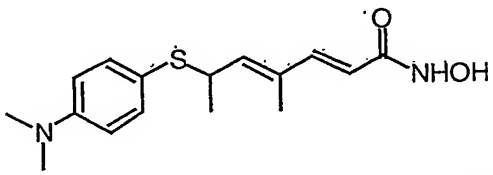
22b

4-(4-Dimethylamino-phenylsulfanyl)-2-methyl-pent-2-en-1-ol



24c

6-(4-Dimethylamino-phenylsulfanyl)-4-methyl-hepta-2,4-dienoic acid ethyl ester



25c

6-(4-Dimethylamino-phenylsulfanyl)-4-methyl-hepta-2,4-dienoic acid hydroxyamide

3. A process for the preparation of a compound of general formula (1), comprising the addition of a compound of general formula (5) to general formula (4), optionally followed by further derivatisation.

4. A process for the preparation of a compound of general formula (1), comprising the addition of a compound of general formula (20) to a compound of general formula (17).

5. A pharmaceutical composition comprising a compound of general formula (1), and optionally a pharmaceutically acceptable adjuvant and/or diluent.

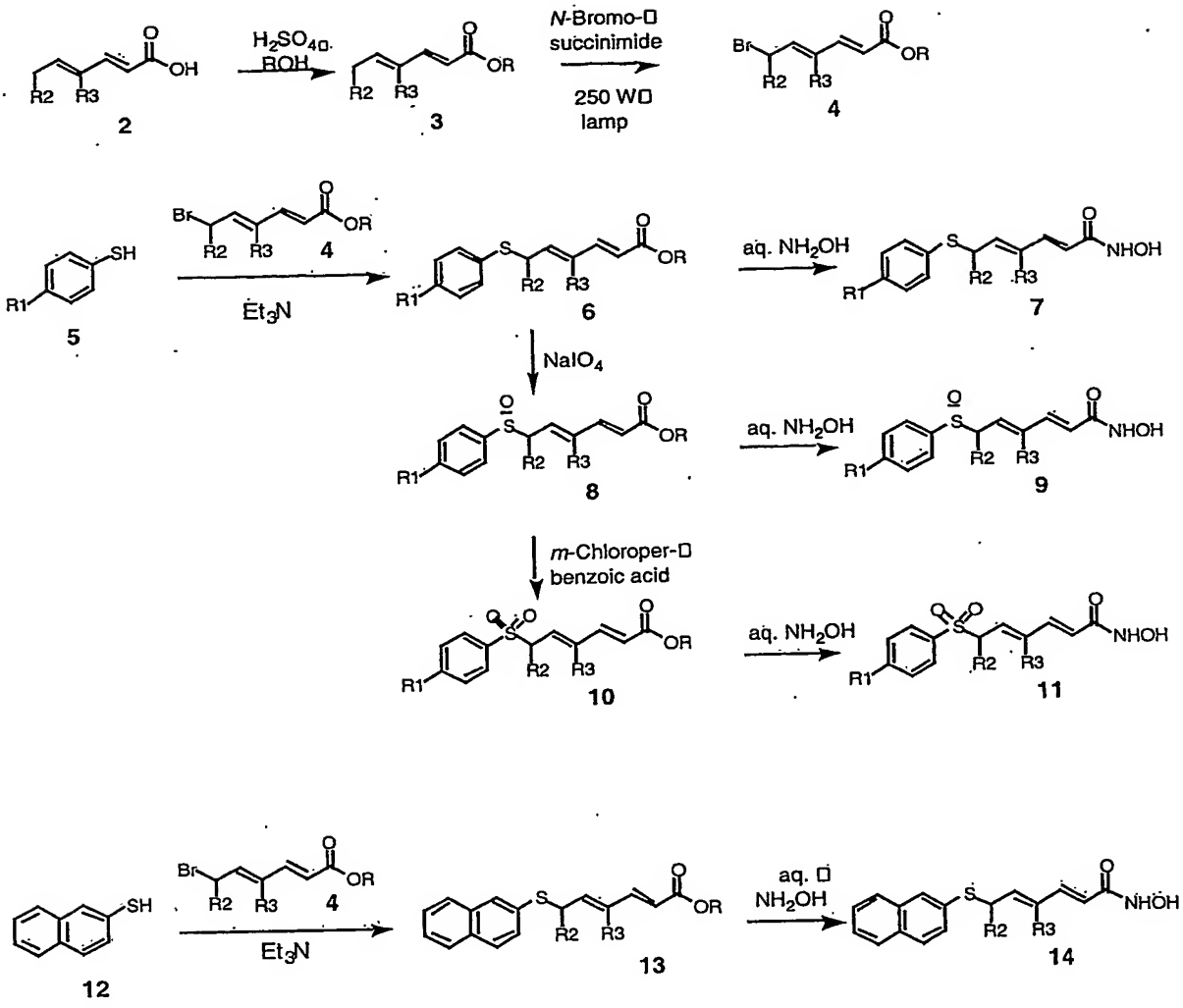
6. A compound of general formula (1) for use in medicine.

7. A method of treatment of an individual suffering from a disease condition, the method comprising administering to the individual a therapeutically effective amount of a compound of general formula (1).

8. A method of inhibition of histone deacetylase activity in an individual suffering from a disease condition, the method comprising administering to the individual a therapeutically effective amount of a compound of general formula (1).

9. The use of a compound of general formula (1) in the manufacture of a medicament for the treatment of cancer.

Scheme 1



	R^2	R^3	R
3a	H	H	Me

	R^2	R^3	R
4a	H	H	Me

	R^1	R^2	R^3	R
6a	H	H	H	HO
6b	Cl	H	H	MeO
6c	H	H	H	MeO
6d	NMe_2	H	H	MeO
6e	OMe	H	H	Me

	R^1	R^2	R^3
7a	H	H	H
7b	Cl	H	H
7c	NMe_2	H	H

	R^1	R^2	R^3	R
8a	H	H	H	MeO
8b	Cl	H	H	MeO
8c	OMe	H	H	MeO
8d	H	H	H	H

	R^1	R^2	R^3
9a	Cl	H	H
9b	OMe	H	H

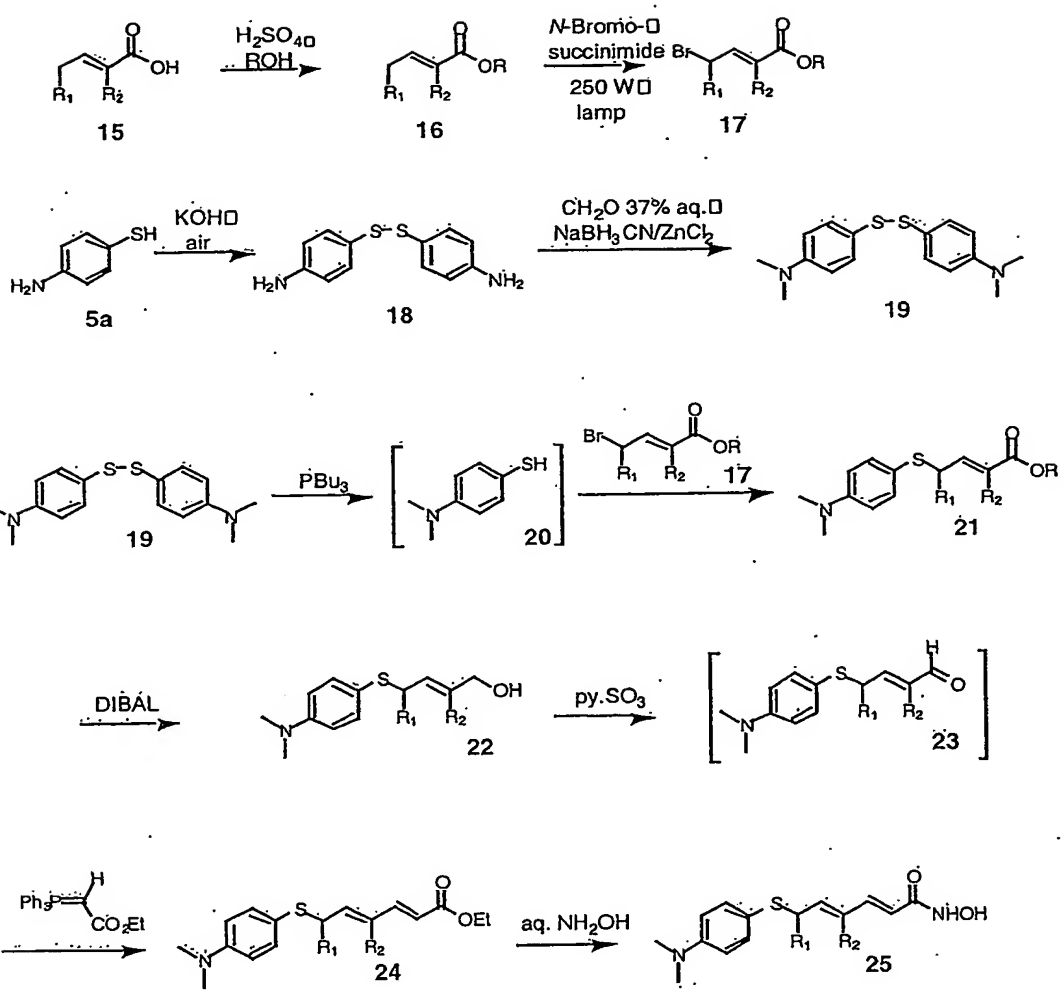
	R^1	R^2	R^3	R
10a	H	H	H	HO
10b	H	H	H	Me

	R^1	R^2	R^3
11a	H	H	H

	R^2	R^3	R
13a	H	H	H
13b	H	H	H

	R^2	R^3
14a	H	H

Scheme 2



	R1	R2	R
16a	H	H	H
16b	Me	Me	Me

	R1	R2	R
17a	H	H	H
17b	Me	Me	Me

	R1	R2	R
21a	H	H	H
21b	Me	Me	Me

	R1	R2
22a	H	H
22b	Me	Me

	R1	R2
24a	H	Me
24b	Me	H
24c	Me	Me

	R1	R2
25a	H	Me
25b	Me	H
25c	Me	Me